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ZINC IMPROVES THE QUALITY OF SEMEN IN ALBINO RATS

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ABSTRACT

BACKGROUND: *Zinc deficiency produces male infertility, causes an impairment of sexual development. Previously, it was thought that this impairment occurred in young animals and produced hypogonadism. More recent investigations showed that adult animals are also affected that result in suppression of germ cell maturation and testes begin to atrophy when the animals are fed zinc-deficient diets for up to 5 wk. Zinc is one of the important antioxidant, ranking second as most abundant tracer element present in the body. Zinc supplementation in semen decreases the activities of reactive oxygen species and enhanced the activities of spermatozoa and increases the quality of semen.*
METHODS: *Forty adult albino rats were selected for the study and divided into two groups the group A, control group and received injection normal saline 1 cc intraperitoneally daily for eight weeks. Group B was received Zinc chloride in a dose of 1mg / kg body weight intraperitoneally daily. On the day of completion of treatment, the animal was sacrificed; the epididymides were excised and used for semen analysis.*
RESULTS: *The mean ± SEM of number of sperm cells/ ml was 7305200 ± 4154514.4 and 16281000 ± 110121.8 in groups A and B respectively. Number of sperm cells / ml in group B was increase significantly (P = 0.002) as compare to groups A. The mean ± SEM of percentage motility of sperms was 72.8 ± 1.59374 and 90.0 ± 1.14018 in groups A and B respectively. Percentage of motility of group B was increase significantly (P< 0.0001) as compare to group A.*
CONCLUSION: *Based on this study, it is, concluded that the zinc increases the motility and numbers of sperms. Zinc, which is an antioxidant protects the germinal epithelium from the toxic effects of heavy metals and other chemicals and restored the most of characteristic features of semen.*

KEY WORDS: Zinc, Semen, Spermatozoa, Albino Rats, Reactive Oxygen Species

INTRODUCTION:

According to the American Society for Reproductive Medicine, one out of every seven couples is affected by infertility [1]. Approximately 85% of couples are able to conceive after one year of unprotected intercourse [2]; however, the 15% who are unsuccessful are considered as infertile. In 30% to 40% of infertility cases, the male factor is a contributing aspect, but in 20% of infertile couples the male factor is the sole cause of infertility [2]. Zinc functions in over 300 enzymatic reactions in the body. It plays a key role in the synthesis and stabilization of genetic material. It is necessary for cell division and the synthesis and degradation of carbohydrates, lipids and proteins. As part of the enzyme copper-zinc superoxide dismutase, zinc helps to protect cells and other compounds against the effects of free radicals. The high concentration of zinc in organs likes prostate, testicles and in the spermatozoa itself suggests its importance in reproduction [3].
The oxygen is in the body required for the aerobic respiration, which is essential for energy production. When the oxygen is use in the body it leads the production of reactive oxygen species (ROS) [4]. When levels of reactive oxygen species (ROS) overwhelm the body's antioxidant defense system, oxidative stress (OS) occurs. OS is a condition in which the elevated levels of ROS damage cells, tissues, or organs [5]. ROS trigger many pathological processes in the male reproductive system, and these processes have been implicated in cancers of the bladder and prostate, as well as in male infertility [4].
Spermatozoa are sensitive to OS because they lack cytoplasmic defenses [6]. The sperm plasma membrane contains lipids in the form of polyunsaturated fatty acids, which are vulnerable to attack by ROS. ROS, in the presence of polyunsaturated fatty acids, triggers

TABLE – I
MEAN* NUMBERS OF SPERMS CELLS / ML IN SEMINAL FLUID OF ALBINO RATS IN DIFFERENT GROUPS AT VARIABLE TIME INTERVAL

Groups	First week (1)	P-Value	Third week (2)	P-Value	Fifth week (3)	P-Value	Eight week (4)	P-Value
A (η = 20)	6656000 ± 396908.0 (η = 5)	A1 VS B1 0.009	6435600 ± 411991.33 (η = 5	A2 VS B2 0.005	7130200 ± 554279.39 (η = 5)	V3 VS B3 0.004	7305200 ± 418020.02 (η = 5)	A4 VS B4 0.002
B (η = 20)	9313200 ± 827205.5 (η = 5)		9252800 ± 279556.83 (η = 5)		11251400 ± 333085.96 (η = 5)		16288000 ± 1101216.8 (η = 5)	

*Mean + SEM (means of five animals)
·P value ? 0.05 means statistically significant.

TABLE – II
MEAN PERCENTAGE OF MOTILITY OF SPERMS IN SEMINAL FLUID OF ALBINO RATS IN DIFFERENT GROUPS AT VARIABLE TIME INTERVAL

Groups	First week (1)	P-Value	Third week (2)	P-Value	Fifth week (3)	P-Value	Eight week (4)	P-Value
A (η = 20)	76.4 ± 1.50333 (η = 5)	A1 VS B1 0.004	75.4 ± 1.50333 (η = 5)	A2 VS B2 0.007	76.2 ± 2.43721 (η = 5)	A3 VS B3 0.003	72.8 ± 1.59374 (η = 5)	A4 VS B4 0.000
B (η = 20)	89.2 ± 1.65529 (η = 5)		86.2 ± 1.52971 (η = 5)		89.8 + 1.52971 (η = 5)		90.0 ± 1.14018 (η = 5)	

* Mean + SEM (means of five animals)
·P value ? 0.05 means statistically significant.

a chain of chemical reactions called lipid peroxidation [7]. ROS can also damage DNA by causing deletions and mutations. There are two main sources of ROS in semen: leukocytes and immature spermatozoa [8]. Oxidative stress and its role in the origins of male infertility were first appreciated in 1943. Human spermatozoa are vulnerable to oxidative stress created by reactive oxygen species (ROS) such as H_2O_2 [9].

Zinc has antioxidant properties that counteract reactive oxygen species (ROS). Thiols, such as glutathione, balance the levels of ROS produced by spermatozoa and influence DNA compaction and the stability and motility of spermatozoa. Zinc, ROS and thiols affect apoptosis, which is important for sperm release, regulation of follicle atresia, degeneration of the corpus luteum and endometrial shedding. Therefore, the concentrations of these nutrients may have substantial effects on reproduction. In conclusion, nutritional and biochemical factors affect biological processes in male and female reproduction [10].

MATERIAL AND METHOD:

Experimental study was conducted at Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi from November 2004 to August 2005.

Forty adult Wistar albino rats were obtained from the animal house of Basic Medical Sciences Institute. Animals were maintained at food and water ad libitum. The animals were divided into two groups, A and B each group consists of 20 animals.

Group A: Served as Control Group was divided into four subgroups A1, A2, A3 & A4 based on period of treatment (1, 3, 5 and 8 weeks) and each subgroup contained five animals. Each subgroup was received injection Normal Saline 1 ml intraperitoneally daily for their respective period of treatment.

Group B: Treated group was divided into four subgroups B1, B2, B3, & B4 based on period of treatment (1, 3, 5 and 8 weeks) and each subgroup contained five animals. Each subgroup was received zinc chloride in dose of 1 mg/kg body weight intraperitoneally daily for their respective period of treatment. [11].

On day of completion of period of treatment the animals were sacrificed, the testis and epididymis were carefully separated. A small nick was given, to cauda epididymis and cut epididymis placed in Petri dish containing 5 ml of normal saline. The cut epididymis was, left for few minutes in Petri dish so that the sperms from epididymis could swim out of epididymis and collected in saline [12].

The number of sperms was counted with

help of Neubauer ruling method. A small drop of fluid from Petri dish was, taken with help of hemoglobin pipette and charged the Neubaur Chamber and number of sperms was counted [13].

For the motility of sperms a small drop of fluid from Petri dish was taken with help of hemoglobin pipette and placed on the centre of glass slide and covered with cover slip and examined under light microscope immediately for the counting motility. A total of 300 sperms were counted for each sample and examine under light microscope at 10X magnification, finally the percentages of immotile were calculated by formula [14].

$$\frac{\text{Number of motile sperms} \times 100}{300}$$

Smear Procedure: a drop of seminal fluid was taken from the Petri dish with help of hemoglobin pipette and placed at one of the edge of glass slide and gently spread over the entire surface of slide. The smears were air dried and fixed in 95% methanol stained with Hematoxylin and Eosin [15]. The level of significance (P) was calculated by the help of student's t-distribution table with the help of which P- value, read against the table degree of freedom (d. f.). The significance level was considered as P ? 0.05

All the calculations were done utilizing computer software, "SPSS 15.0" in Window 2000 XP.

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OBSERVATIONS AND RESULTS:

The present study was designed to observe the beneficial antioxidant effects of zinc on testicular gremial epithelium of Albino Rats. The observations and their results on microscopic examinations of semen were observed and compared with control groups and previous studies.

The method of counting the numbers sperms is shown in figure-1. Mean values of numbers of sperms were recorded and shown in table-I. The mean ± SEM number of sperm cells / ml in groups A and B after eight week of treatment was 7305200 ± 418020.02 & 16281000 ± 1101216.8 respectively. Number of sperm in group B were increase as compared to group A there was significant increase (P = 0.002) in number of sperms.

The method of counting of motility of

FIGURE 1

PHOTOMICROGRAPH SHOWING METHOD OF COUNTING OF NUMBERS OF (SZ) IN SEMINAL FLUID WITH HELP OF NEUBAUER RULING METHOD, AT LOW MAGNIFICATION

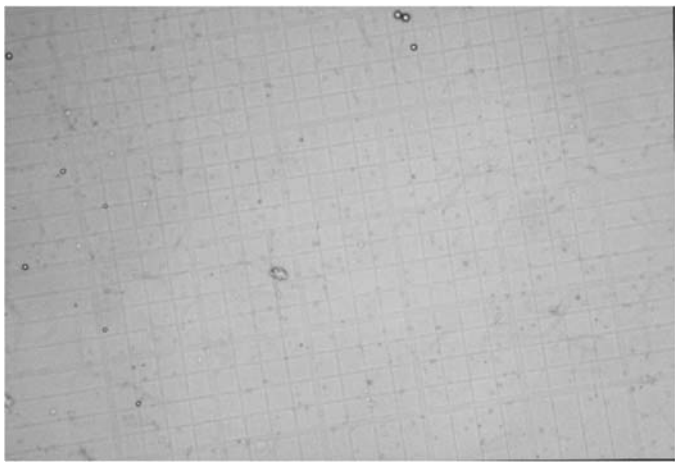


FIGURE 2

PHOTOMICROGRAPH SHOWING METHOD OF MEASURING OF PERCENTAGE OF MOTILITY OF SPERMATOZOA (SZ) IN SEMINAL FLUID, AT HIGH MAGNIFICATION

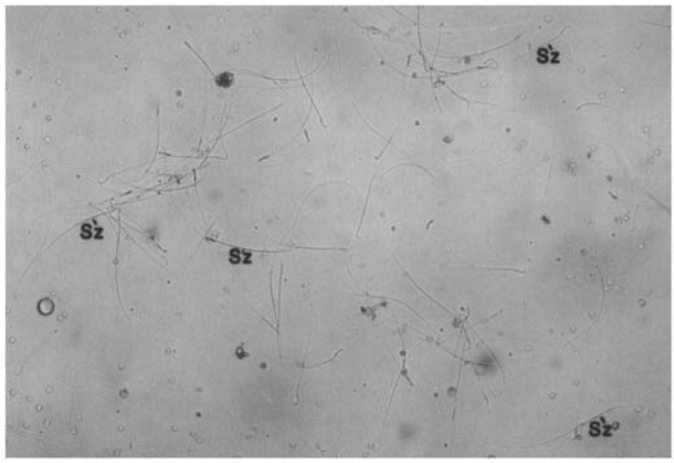
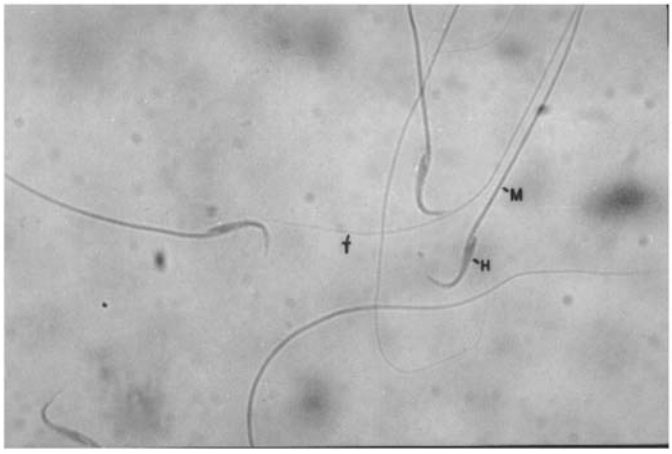


FIGURE 3

PHOTOMICROGRAPH OF HEMATOXYLIN AND EOSIN STAINED OF SMEAR OF SEMINAL FLUID, SHOWING MORPHOLOGY OF RAT'S SPERMATOZOON WITH TYPICAL HOOKED HEAD (H), PROXIMAL WIDER MID-PIECE (M) AND DISTAL THIN TAIL (T), AT HIGH MAGNIFICATION



sperm's is shown in figure-2. While counting of percentage of motility of sperms no was stain used just one drop of seminal fluid placed on glass slide. Mean ± SEM percentage of motility of sperms in seminal fluid of albino rats in groups A & B at eight of treatment were 72.8 ± 1.59374 & 90.0 ± 1.14018 respectively. While comparing percentage of motility of sperms in group B with group A there was statically significant increase ($P < 0.0001$) in motility of sperms

DISCUSSION:

The results revealed that supplementation of improved the semen quality in the treated group as compared to the control group. Zinc (Zn) plays an important role in polymeric organization of macromolecules like DNA and RNA, protein synthesis and cell division [16]. Zinc has a role in prostate, epididymal and testicular functions [17]. Zinc has been reported to influence the process of spermatogenesis, controls sperm motility, stabilizes sperm membrane, preserves the ability of sperm nuclear chromatin to undergo decondensation and modulates sperm functions [18].

The results revealed significantly higher values of semen quality in groups supplemented with Zn as compared to the control group of albino rats. The present results are in agreement with the findings of Nishant K & Coworker (2006) [19] and researchers who observed increased semen volume when they supplemented Zn sulfate in the diet of goats and rabbits [20].

Semen volume mainly constitutes secretion of the testes, epididymis and accessory sex glands, especially prostate gland. Zn has been reported to stimulate growth and development of primary, secondary and accessory sex organs as evidenced by atrophy of these organs in rams, when they were fed a Zn deficient diet. The main source of zinc in the semen is the prostate gland where the highest concentration of Zn has been demonstrated, and it acts as a marker of prostatic functions [21].

In the present study, a significant ($P = 0.002$) increase in sperm concentration and sperm number per ejaculate in all the Zn-supplemented groups as compared to the control group, this indicated a beneficial effect of Zn on spermatogenesis. Similar results were observed in men, rams, bucks and rabbits [22], when Zn supplemented in their diet. This may be due to the fact that Zn plays an indispensable role in spermatogenesis [21]. All these factors may account for improved sperm concentration and sperm number per ejaculate in Zn-supplemented groups. Zn controls the motility of sperm by controlling energy utilization through the ATP system, through regulation of phospholipids energy reserves and improving sperm oxygen uptake [22]. Supplementation of Zn revealed significant improvement in both number and individual motility in all Zn supplemented groups as compared to the control group. Results were in agreement with previous reports in men, sheep and rabbits [20]. Improved sperm motility may be due to the fact that the primary donor of energy needed by the sperm flagella for movement is ATP and Zn controls the motility of sperm by controlling energy utilization through the ATP system [22]. Zn is also a scavenger of free oxygen radical and protects sperm from oxidative damage and lipid per oxidation by inhibiting phospholipase [21]. Thus, the anti-oxidant action of Zn may be responsible for improved motility of sperm in Zn-supplemented groups.

CONCLUSION

It may be concluded that supplementation of Zn in the diet improved semen quality in terms of quantitative and qualitative characteristics of semen, as compared to the non-supplemented control group. Zinc affects the actively meiotic cells that mean that the zinc acts at the genomic level.

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